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NEW APPROACHES TO PREPARATION AND STORAGE OF PLATELETS

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ABSTRACT

During the last century, transfusion of blood components has become a prerequisite for treatment in many clinical settings. The availability of plastic bags and methods for centrifugation and separation of blood cells, has enabled processing of whole blood into blood cell components, and made it possible to optimize transfusion therapy, as well as storage conditions. Automation of the processes has been introduced, which increases reproducibility and standardization of the prepared components.

Recommendations of leukocyte reduction to minimize side-effects caused by concomitant leukocytes have resulted in development in different types of filters.

Today, universal leukocyte reduction is practice in many countries and in Sweden, a majority of the blood components are leukocyte reduced.

For the immunosuppressed patients, who are at risk of developing transfusion associated graft-versus host disease attributed to passenger T-lymphocytes, there is need for further treatment of the blood components to restrain the proliferation of T-lymphocytes, and hence prevent TA-GVHD. The prevalent method has been gamma irradiation, and in recent years X-ray irradiation has been available as another alternative to gamma irradiation from radioactive sources.

The studies included in the present thesis focus on 1) preparation techniques for leukocyte reduction of whole blood which saves platelet, and results in preparation of erythrocytes, plasma and platelets, 2) automated preparation of leukocyte reduced platelets from pooled buffy coats and standardization of platelet content by selection of buffy coats, 3) evaluation of platelet additive solutions with different composition, with the aim to optimize storage conditions and 4) effects of gamma- and X-ray irradiation on platelets during storage.

The results indicate that leukocyte reduction of whole blood with a filter that saves platelets results in blood components with sufficiently low leukocytes, satisfactory recovery of erythrocytes and platelets, and high quality as measured by in vitro parameters, which were maintained during storage.

Automated preparation with OrbiSac of leukocyte reduced platelets from pooled buffy coats and standardization of platelet content by selection of buffy coats was introduced. Prepared platelets displayed in vitro characteristics equivalent to the previous manual method, during storage for 7 days. Moreover, the platelet count variation was reduced.

Evaluation of different platelet additive solutions concluded that addition of phosphate is beneficial for storage of platelets. This finding has had an impact on development of the next generations of platelet additive solutions.

Effects of different irradiation equipments were studied. No major deleterious effects of gamma irradiation on platelets during storage were identified. For X-ray irradiation previous data are scarce, especially for platelets, and our results imply that X-ray irradiation does not compromise the quality of platelets during storage for 7 days.

The studies all represent new options for blood component preparation, which has enhanced efficacy and safety.

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- I Larsson S, Gulliksson H, Paunovic D. Evaluation of a whole-blood WBC-reduction filter that saves platelets: in vitro studies.
Transfusion 2001;41:534-539.
- II Larsson S, Sandgren P, Sjödin A, Vesterinen M, Gulliksson H. Automated preparation of platelet concentrates from pooled buffy coats: in vitro studies and experiences with the OrbiSac system.
Transfusion 2005;45:743-751.
- III Gulliksson H, Larsson S, Kumlien G, Shanwell A. Storage of Platelets in Additive Solutions: Effects of Phosphate.
Vox Sang 2000;78:176-184.
- IV Larsson S, Sandgren P, Källman A-S, Lundahl J, Gulliksson H. Irradiation of platelets for transfusion with two different techniques: Irradiation with cesium and X-rays.
Submitted (Transfusion).

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LIST OF ABBREVIATIONS

ACD	Acid-citrate-dextrose
ADP	Adenosine diphosphate
ATP	Adenosine triphosphate
BC(s)	Buffy coat(s)
BC-PC	Buffy coat (derived) platelet concentrates
CCI	Corrected count increment
CPD	Citrate-phosphate-dextrose
CFU	Colony forming unit
DEHP	Diethylhexyl phthalate
eBDS	enhanced Bacterial Detection System
ESC	Extent of Shape Change
FNHTR	Febrile Non Hemolytic Transfusion Reaction
GP	Glycoprotein
HLA	Human leukocyte antigen
HPA	Human platelet antigen
HSR	Hypotonic shock response
IL	Interleukin
LDH	Lactate dehydrogenase
MPV	Mean platelet volume
NK-cells	Natural killer cells
PAS	Platelet additive solution
PC(s)	Platelet concentrate(s)
PF4	Platelet factor 4
PLT	Platelet
PRP	Platelet rich plasma
PTS	Pneumatic tube system
PVC	Polyvinyl chloride
RANTES	Regulated on Activation, Normal T-cell Expressed and Secreted
RBC	Red blood cell
SAGMAN	Saline-adenine-glucose-mannitol
SD	Standard deviation
TA-GVHD	Transfusion associated Graft versus Host disease
TCA	Triboxylic acid cycle
TNF- α	Tumor necrosis factor-alpha
vWF	Von Willebrand Factor
WB	Whole blood
WBC	White blood cell

1 INTRODUCTION

1.1 PLATELET PREPARATION-SHORT HISTORICAL BACKGROUND

Blood component preparation and component therapy was a new concept made possible by development of the plastic blood container in the 1950s, as well as techniques and devices which allowed centrifugation and separation. Until then, transfusion of whole blood was the prevailing method (1). Duke reported already 1910 the importance of platelets to stop bleeding, although at that time fresh whole blood was transfused (2).

Platelet rich plasma prepared by centrifugation of whole blood was the first available platelet concentrate (PC). Four to six units transfused were considered as an adequate platelet dose to an adult patient. During the decades to come, increasing knowledge about platelet metabolism, development of gas permeable platelet storage containers and additive solutions, has resulted in platelets with maintained quality during 5-7 days of storage as shown by several in vitro and in vivo studies. The application of methods to reduce leukocytes and inactivate lymphocytes and pathogens without compromising the quality of platelets, have increased the safety for transfusion recipients.

1.2 PLATELET BIOLOGY AND FUNCTION

Platelet are small anucleate cell fragments, with a diameter of approx. 2µm, with a medium volume of 7-9 fL in the resting state, formed as a small disc. Due to light refraction by their discoid form, the “swirling” phenomenon is seen, which is often used as a gross measure of platelet viability (3). The number of circulating platelets in healthy individuals shows a great variation, with a concentration of $150-400 \times 10^9/L$, and a life span of 7-10 days. Platelets originate from megakaryocytes, formed in the bone marrow, derived from hematopoietic stem cells further developed in response to different growth factors and cytokines. Thrombopoietin, produced primarily in the liver, is the principal regulator of megakaryocyte development and platelet production. ABO-, HLA class I and human platelet antigens (HPA) are expressed on the surface, and may all be the target of alloantibodies, resulting in refractoriness to transfusion.

Intracellularly, the platelets have two network of internal membranes, the open canicular system and the dense tubular system. The platelet also contain different types of granula: α -granules, dense bodies and lysosomes, where the α - granules contain adhesive proteins, e.g. fibrinogen, fibronectin, thrombospondin and von Willebrand Factor, vWF, and the dense bodies is a source of different activating substances, as adenosine diphosphate (ADP), serotonin and calcium.

Upon activation, the granulae form a continuum with the open canicular system and releases its content of adhesive and procoagulant substances, exposing new antigenic determinants on the surface, which initiates and augments the hemostatic process. Also present in the cytoplasm, are the mitochondria, which have importance for the energy production. Figures 1-4 shows the ultrastructure and different shapes of platelets.

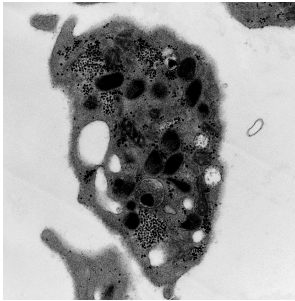


Figure 1. Transmission electron microscopy (TEM) image showing normal ultrastructure including mitochondria, α -granules, dense granules and glycogen in clusters from a fresh platelet at day 1. (Per Sandgren, Kjell Hultenby)

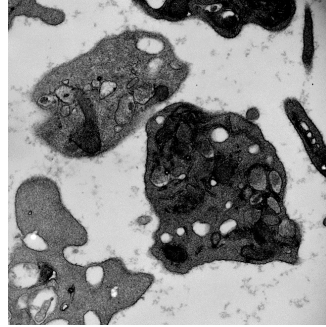


Figure 2. Transmission electron microscopy (TEM) image from a platelet at day 7 showing lucent granules and very few glycogen particles in the cytoplasm. (Per Sandgren, Kjell Hultenby)

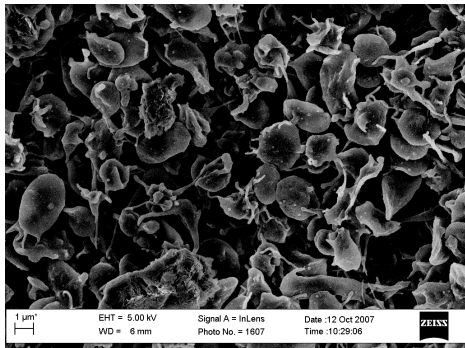


Figure 3. Scanning electron microscopy (SEM) picture of discoid platelets in a BC platelet concentrate, stored for 1 day in PAS-II. (Per Sandgren, Kjell Hultenby)

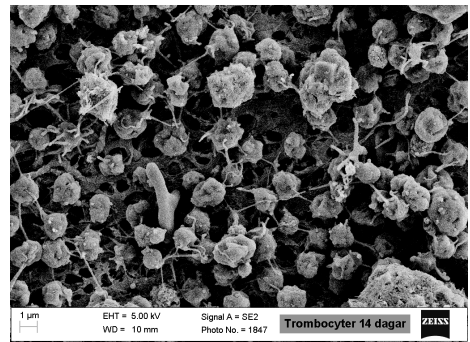


Figure 4. Scanning electron microscopy (SEM) picture of spherocytical platelets in a BC platelet concentrate, stored for 14 days in PAS-II. (Per Sandgren, Kjell Hultenby)

The platelets play a crucial role in primary hemostasis, i.e. the initial formation of a hemostatic plug, at the site of a damaged endothelium, but also for initiating and augmenting the coagulation process.

The triggering event is a vascular injury, which exposes subendothelial collagen and vWF, to which platelets adhere by binding of the GpIb α , which by intracellular signaling leads to degranulation of substances that mediates further adhesion and aggregation of platelets, and subsequent stabilization of the hemostatic plug by fibrin polymerization.

In addition, platelets has been found to exert many other important functions, as in tissue regeneration (4-9), due to the secretion of growth factors, which is applied in various clinical settings, and as part of the immune system (10-12).

1.3 PREPARATION OF PLATELETS

Preparation of platelets is done either with whole blood as source or by apheresis technique. The platelets can be suspended in concomitant plasma from the donors, or in a combination of plasma and platelet additive solution, and thereby will be influenced by the anticoagulant used. For whole blood donations, CPD is used, whereas ACD is commonly used in apheresis, which will be discussed in later sections.

1.3.1 Preparation of platelets from whole blood

1.3.1.1 Separation of blood cells by centrifugation

Separation of blood cells by centrifugation is based on their sedimentation rate, according to their size and density, see Table 1. If anticoagulated blood is left in a blood bag, the cells will sediment due to gravity. To make this process faster, centrifugation is applied, the process being accelerated by higher g-force. The erythrocytes will sediment to the bottom of the bag, whereas leukocytes and platelets will settle on the top of the erythrocytes.

During centrifugation, erythrocytes and leukocytes sediment faster, due to their higher density, whereas platelets sediment slowly. If the centrifugation is stopped at a point where erythrocytes and leukocytes have sedimented, but the platelets are still present in the plasma, platelet-rich plasma can be recovered. On the other hand, if centrifugation is continued long enough for the platelets to sediment, the platelets are instead recovered within the buffy coat. Both g-force and time of centrifugation will determine the separation of cells.

Table1. Density of blood cells and plasma Ref: Guide to the preparation, use and quality assurance of blood components, 16th ed., Council of Europe 2010

	Mean Density (g/mL)	Mean Volume (10^{15} L; fL)
Plasma	1,026	
Platelets	1,058	9
Monocytes	1,062	470
Lymphocytes	1,070	230
Neutrophils	1,082	450
Red cells	1,100	87

1.3.1.2 Preparation of platelets from platelet-rich plasma

For platelets (PLTs) recovered from whole blood donation, the platelet rich plasma (PRP) method was the first to be practiced: the whole blood, kept at 20-24°C, for a maximum of 24 hours before preparation, is subjected to a relatively short, low g-force centrifugation (“soft spin”, up to 1000 g) leading to sedimentation of the erythrocytes and leukocytes, whereas the platelets are captured within the plasma fraction. In a second centrifugation step of the PRP, with a greater g-force (“hard spin”, approx. 3000 g), the platelets are pelleted to the bottom of the bag and the major part of the cell free supernatant plasma is extracted. Subsequently, the platelets are resuspended in the retained volume of plasma (40-70 ml). The platelet content is variable, due to variations in donor platelet concentration, and in preparation. Platelet content shall meet the quality criteria according to European regulations, i. e. 200×10^9 PLTs/ unit, which usually corresponds to 4-6 pooled units, or a minimum of 60×10^9 PLTs/single unit (13). Regulation in USA requires a minimum of $5,5 \times 10^9$ PLTs/unit (14). Preparation of PRP is the main method to produce PC from whole blood in USA.

1.3.1.3 Preparation from buffy coats

A principally new method was introduced in the 1980s (15), where the whole blood was subjected to an initial centrifugation with high g-force (“hard spin”, approx 3000 g), which forces all cells, i. e. erythrocytes, leukocytes and platelets to sediment according to their relative density, resulting in a thin layer of leukocytes and platelets on top of the erythrocytes. The thin layer of leukocytes and platelets, barely visible for the eye, called the “buffy coat” (BC) layer, consists of approx. 70-90% of leukocytes and up to 70-80% of the platelets from the original whole blood donation (16-18). The BC is separated into a bag in a following preparation step, by automated separation technique or by manual press, together with an amount of plasma intended for suspension of the platelets. The platelets are extracted from the buffy coat bag by an additional centrifugation (“soft spin”) resulting in sedimentation of the leukocytes, and a platelet-rich plasma supernatant which is transferred to a platelet storage container. Initially, single units of buffy coat platelets were prepared (15), followed later by a method with pooling of BC, usually 4-6 BCs (16), to increase the yield and the centrifugation conditions. This results in a platelet unit with an adequate dose for an adult patient ($>200 \times 10^9$ PLTs/unit)(13).

Preparation of BC-PC is practiced in many European countries. In Sweden it accounts for approx. 70 % of the annual production (19), and at our clinic the proportion is approx. 85%.

During the last decade, different equipment for automation in preparation of platelets from buffy coats has been developed, i.e. the OrbiSac Buffy-coat system (Terumo BCT, former Caridian BCT), and the TACSI system (Terumo BCT).

The OrbiSac Buffy-coat system, which is evaluated in this thesis (Paper II), and by other authors (20-25), will be addressed in later sections. The Terumo Automated Centrifuge and Separation Integration (TACSI) system that was introduced in 2007, which produces six platelet units per process, has also been validated (26, 27).

Devices to automate almost all processing steps for blood component preparation from whole blood have been developed, the Atrous system (Terumo BCT, former Caridian

BCT) being the first example. The Atrius system can be configured into different protocols, among which BC can be obtained (protocol 2C+)(28-30). The most recent configuration (protocol 3C) produces one unit of red blood cells (RBC), one unit of plasma and one unit of platelets per process, which results in platelets unit that may be transfused as single, or pools of 4-6 units.

1.3.2 Platelet prepared by apheresis

Platelets prepared by apheresis technique was introduced in the 1960s (31), and the development has led to apheresis equipments with continuous flow-devices with plastic disposable sets, using single-needle access and in-process systems for leukocyte reduction (32). With apheresis technique, whole blood is drawn and mixed with anticoagulant, and separated into cell fractions due to their relative density by centrifugation, the platelet-rich plasma fraction is collected into a platelet storage bag, and the remaining cells and most of the plasma is returned to the donor. In this way, it is possible to obtain a larger number of platelets from a single donor, sufficient for up to 4 transfusion doses, which is a advantages when platelets of a certain type is required, e.g. HLA-matched or HPA1a-negative. Also, the patient is exposed to fewer donors, with less risk of viral transmission.

1.4 STORAGE OF PLATELETS

The demand for platelets for transfusion are steadily increasing over the years, and readily accessible platelets are needed in many clinical situations, such as massive bleeding in conjunction with trauma, transplantation etc.

Over the years, increasing knowledge about platelet metabolism, effects on storage at different temperatures and the development of new plastic materials and storage solutions has made it possible to prolong the shelf life of stored platelets, with preservation of haemostatic effect.

As for whole blood, storage in the refrigerated state was initially practiced also for storage of platelets, but was found to lead to rapid clearance after transfusion. Platelets refrigerated for more than 12 to 24 hours do not survive long in the recipients' circulation (33). Storage of PRP and PC at room temperature was reported to result in preserved viability during longer storage periods (34, 35), although a number of abnormal in-vitro parameters were shown at room-temperature, due to the higher metabolic activity. However, in-vivo studies that were performed of room-temperature stored platelets showed that they circulated longer in the recipients compared to chilled platelets (7 to 9 days vs. 2 to 4 days) and demonstrated better in-vivo function (sustained correction of bleeding time) after prolonged storage in the blood bank (36, 37).

The mechanisms for the rapid clearance of chilled platelets have emerged over the last decade. In brief, short-term cooling causes clustering of von Willebrand factor complexes on the platelet surface, rendering them susceptible to ingestion by hepatic macrophages that recognizes them via $\alpha_M \beta_2$ integrin receptors (38-40). Longer storage results in emergence of a different neopeptide recognized by hepatocyte asialoglycoprotein receptors, leading to clearance.

In studies of storage of platelets at 4°C for a period of 21 days, in-vitro characteristics for metabolic and cellular characteristics were maintained to a great extent (41, 42). Ongoing studies seek to alter these neoepitopes, in order to prevent the rapid clearance. Until such a solution is found, cold storage of platelets for transfusion is not feasible.

Comparison of storage of platelets at room temperature and at body temperature performed by Holme and Heaton shows metabolic benefits for room temperature storage, and report that platelet ageing during storage at 22°C is reduced by 56-58% compared to that at 37°C (43). Storage temperature above 24°C appears to be more disadvantageous, due to accumulation of substances as a result of the increased metabolic activity (34). Based on these data, the currently recommended storage temperature for platelets is 20-24°C (13).

Platelets derive energy by two different pathways: anaerobic glycolysis and oxidative metabolism of plasma free fatty acids, their relative contribution to the total energy production being 15% and 85%, respectively. Studies of platelets stored at 22°C show that even in presence of oxygen, a low level of anaerobic glycolysis occurs, where one molecule of ATP per lactic acid molecule is formed, the latter buffered primarily by bicarbonate from the plasma (44). Glycolysis is up-regulated as much as sevenfold when O₂ levels decline, and hence accelerating the production of lactic acid. Plasma bicarbonate is gradually consumed during storage, which eventually leads to a fall in pH caused by unbuffered lactic acid. At a pH below 6.4, the platelets begin to swell, change form from disc to sphere, activate and lyses (45). When medium pH falls below 6.2 the majority of platelets is severely damaged, and fails to circulate. Platelet units with high platelet content require more O₂ and produce more lactic acid, especially when O₂ levels decline, in the absence of a corresponding amount of plasma bicarbonate.

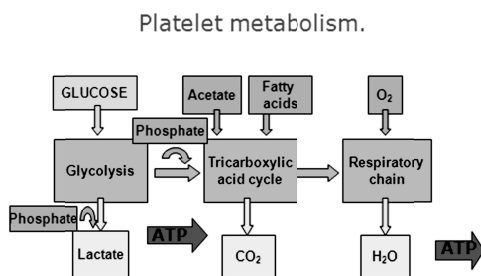


Figure 5. Anaerobic and aerobic platelet metabolism (Hans Gulliksson)

When this was recognized, in the 1980s, more gas-permeable containers for platelet storage were developed, made of polyolefin, PVC plasticized with tri-(2-ethylhexyl) trimellitate (TEHTM) or butyryl trihexyl citrate, and thinner bags made of PVC with DEHP plasticizers (44). These second-generation containers are up to twice as O₂ permeable as the first-generation containers, although for platelet units with very high platelet content they may not provide an adequate O₂ supply.

With regard to the O₂ supply, another important factor is the surface area of the storage container, available for gas exchange. When platelet units are stored on agitators, they shall be placed freely, and not be allowed to overlap or be covered to a great extent with labels or other material that diminishes O₂ diffusion. The suspension medium is also important, both with regard to buffering capacity and for the volume needed to move the platelets freely within the container.

In 1960s, when PRP-PCs were stored, agitation was considered necessary for keeping platelets in suspension, allowing interruption only during transportation, according to AABB standards up to 24 hours as a maximum. Earlier studies showed a metabolic shift to glycolysis in platelet units when agitation was interrupted (43, 46-48). Agitation may ameliorate hypoxic or toxic metabolic microenvironments and facilitate O₂ use by mitochondria, and may also prevent platelet contact-mediated metabolic changes. Later studies of PRP-PC and apheresis platelets suggest that agitation may be interrupted for up to 30 hours without significant negative effects on in-vitro parameters during storage of 5 days (49, 50). The form of agitation is also of importance, e.g. flatbed agitators appears to cause less damage than elliptical end-over-end and Ferris-wheel rotators (46, 51).

Prolonged storage of platelets at room temperature raises concerns for bacterial growth leading to septicemia, one of the main risks of transfusion (52). This has limited the storage time to 5 days in most countries, and in Germany to 4 days. In Sweden, a prolonged storage time to 7 days is allowed, provided bacterial testing has proved to be negative.

Bacterial contamination derives from small amounts of bacteria entering via the phlebotomy site of the skin, due to insufficient disinfection, or due to disconnection of the closed system at any point of the preparation process. Detection of bacteria is complicated due to the fact that the initial inoculums are often very small (1-10 CFU/ml). In the first hours, the bacteria can be neutralized by the antibacterial properties of blood, but if not, they can grow rapidly and reach high concentration at the end of the storage time. Therefore, the prevalence of bacteria detected will depend on the time of sampling, the volume sampled and the sensitivity of the method used (53, 54). Disinfection of the skin at the venipuncture site, and diversion of the first portion of blood has reduced the risk of bacterial contamination. However, additional screening of bacteria is essential (55). Different methods for bacterial screening have been developed, e.g. BacT/Alert system (bioMerieux, Durham, NC) and eBDS (PallCorp, East Hills, NY), and recently, a number of rapid tests intended for testing immediately prior to issue of the platelets (56, 57).

1.5 PLATELET ADDITIVE SOLUTION

Platelet additive solutions (PAS) have been developed since the 1990s, as a substitute for plasma. The main reasons for using PAS are to reduce the amount of plasma, which is an advantage in case of ABO-incompatibility, and to prevent adverse reactions and circulatory overload, retrieve plasma for fractionation, improve storage conditions and enable photochemical treatment in pathogen reduction (58). Extended storage of platelets is possible, due to the fact that aging of platelets at 22°C is significantly slower than at 37°C.

Important factors for optimizing the storage conditions have proved to be 1) reducing the activation of platelets during whole blood collection, preparation and storage of platelets, 2) reducing the metabolic rate in terms of glucose consumption and lactate production, and 3) ensure an adequate supply of glucose in the storage medium throughout the storage period (59).

The use of PAS makes it possible to add substances which affect platelets. A number of effects have been observed that can be assigned to different chemical compounds (59-61).

By reducing the activation of platelets and improving the metabolism and function by addition of key components in the storage medium, i.e. acetate, citrate, glucose, potassium and magnesium, it is possible to optimize platelet storage conditions.

The compositions of different PASs are presented in Table 2. The first generations of PASs, PAS-I and PAS-II, was followed in the last decade by modifications of PAS-II; called PAS-IIIM.

Table 2. Composition of platelet additive solutions, including commercial names

Ref: Blood component preparation, From benchtop to bedside, Ed. Blajchman, M., AABB Press 2011, Chapter Containers and Additive Solutions for Platelets, Gulliksson, H.

	Plasmalyte A	PAS- I	SetoSol	PAS-II T-Sol, SSP	PAS-III InterSol	CompoSol	PAS-IIIM SSP+
Na Cl	X	X	X	X	X	X	X
KCl	X	X	X	-	-	X	X
MgCl ₂	X	-	X	-	-	X	X
Na ₃ -citrate	-	X	X	X	X	X	X
NaH ₂ PO ₄ / Na ₂ HPO ₄	-	X	X	-	X	-	X
Na-acetate	X	-	X	X	X	X	X
Na-gluconate	X	-	-	-		X	-
D-glucose	-	-	X	-		-	-
Maltose	-	-	X	-		-	-
D-mannitol	-	X	X	-		-	-

Plasmalyte A: Baxter International, Deerfield, IL, USA

SetoSol: Terumo, Kanagawa, Japan

T-Sol, InterSol : Fenwal, La Chatre, France

SSP, SSP+: MacoPharma, Lille, France

InterSol: Cerus, Concord, CA/Fenwal, Lake Zurich, IL, USA

CompoSol: Fresenius Kabi, Bad Homburg, Germany

The first generation of PAS was used in combination with the preparation of platelets from pooled buffy coats. The PAS consisted of a glucose-free medium combined with 25% of plasma (62), and in-vitro and patient transfusion studies demonstrated a high quality. Further development were presented in subsequent studies (17, 63, 64).

An investigation of storage of platelets in a pure saline medium, intended to serve as a reference to PAS, was performed 1992 (65), which unexpectedly proved to have similar in-vitro characteristics. This finding led to the introduction of saline as a PAS, in combination with 40% plasma, as a contributor of glucose and bicarbonate, intended

for storage of platelets from pooled BC for 5 days. This solution was used for several years, until it was replaced by second-generation PAS, PAS-II.

PAS-II was composed of saline, with the addition of citrate at a reduced concentration compared to plasma, and addition of acetate, in subsequent steps, which allowed studies of the effects on platelet metabolism and quality during storage (65). PAS-II is still in clinical use in many countries, and was used for the preparation of the platelets at the time when the studies included in this thesis were performed. BC-PCs suspended in PAS-II are commonly pooled from 4-6 donors, with approx. 35-40% plasma carry-over from the individual BCs, and approx. 60-65% PAS-II.

PAS can be used in combination with preparation of apheresis platelets as well, where the storage medium consists of a combination of plasma (20-40%) and PAS (60-80%). Apheresis equipment are generally designed for the collection of a relatively larger volume of plasma as suspension medium for the platelets, but may be adjusted to collect a more concentrated platelet product in a smaller plasma volume, which subsequently can be mixed with PAS (66, 67).

The PRP method is generally not combined with the use of PAS for routine preparation, although a study showing satisfactory preservation of platelet quality using this concept has been performed (68).

Activation of platelets, caused by the different processing steps and storage has been long known (46, 69-71), measured by a number of different markers. In a comparative study of activation by three different preparation methods, activation of PRP-PC was found to be significantly higher than in BC-PC, whereas apheresis platelets showed intermediate activation (72).

Presence of glucose during the storage period is crucial for platelet metabolism. Due to manufacturing difficulties (i.e. caramelization of glucose at neutral pH during the steam sterilization process), the majority of PAS formulas are non-glucose containing. The glucose is instead derived only from the plasma fraction, leading to low initial glucose levels of 5-10 mmol/L. Effects observed after depletion of glucose is rapid decrease of ATP and cessation of lactic acid production, leading to disintegration of platelets (73),(65). A slight correlation with in-vivo viability in terms of recovery below 50% of transfused platelets occurs at ATP-levels below $4.0 \mu\text{mol/L}/10^{11}$ platelets (74), levels often seen after depletion of glucose. In contrast to plasma, the fall in pH during storage of platelets in PAS will stop at significantly higher level than about 6.0, as a result of the limited amount of glucose. Because the buffering capacity of PAS generally is very limited compared to plasma, they are more susceptible to increased production of lactic acid (74-76). Units with high platelet concentration have an extra risk of glucose depletion at the end of the storage. Recently, studies have been performed of platelets stored in new formulas of PAS, where glucoses is connected to the PAS-container after the sterilization process, with in-vitro results similar to those in the plasma reference (77, 78).

Acetate is used as a substrate for the aerobic platelet metabolism, which enters the tricarboxylic acid cycle and is further oxidized in the respiratory chain of the mitochondria (79, 80). End products are carbon dioxide and water. By formation of bicarbonate from the carbon dioxide produced by acetate, pH can be maintained at a

stable level during storage. The hydrogen ion necessary for bicarbonate production can be derived from lactic acid. In this way, the hydrogen ions produced by glycolysis are balanced by the removal of hydrogen ions by oxidation of acetate. Acetate present in PAS has also been shown to reduce the production of lactate and increase oxygen consumption by platelets (75, 76, 81, 82).

Citrate is included mainly for anticoagulation, but there is some evidence that citrate and magnesium also modifies potassium efflux through the membrane (83). In addition, increased responsiveness to activating agents, i.e. ADP (84), and effects on glucose metabolism has been observed (82), suggesting that PAS should preferably should include low concentrations of citrate to avoid excessive lactate production and low pH. On the other hand, lactate production can be balanced by addition of acetate. A minimum level of 8 mmol/l of citrate seems to be required to prevent clotting problems (85).

The effects of phosphate during storage are as a stimulant of glycolysis to produce lactate, and as a buffer to prevent fall in pH (75, 86). These two effects are counteracting and may neutralize each other.

The presence of phosphate seems to be a critical factor to avoid low adenine nucleotide levels, and may have a significant influence on platelet metabolism when PASs are used. Studies reflecting this are included in Paper III.

Magnesium, which is included in the latest generation of PASs, has been shown to have effects on platelet membrane function, platelet activation and the rate of glycolysis. Increased concentrations of extracellular magnesium ions significantly inhibits exposure of P-selectin, decreases binding of fibrinogen to ADP-activated platelets, and decreases agonist-induced aggregation (87, 88).

Potassium, in combination with magnesium, has been shown to have a beneficial effect on platelet activation (89).

1.6 LEUKOCYTE REDUCTION OF PLATELETS

Leukocytes are generally regarded as contaminants in the blood components prepared from whole-blood, associated with a number of adverse effects. Leukocyte reducing filters with efficient removal of $4\log_{10}$ or more has been developed, making it possible to prepare blood components with remaining leukocytes, with retaining a sufficient amount of the therapeutic blood elements (RBC or PLTs), according to current guidelines (13).

During the 1990s the praxis of leukoreduction was widely spread, and many European countries implemented universal WBC reduction. In Sweden, universal leukoreduction is not mandatory, but is practiced at the majority of Blood Banks, and for 2011 all transfused platelets were leukocyte reduced (vs. approx 90% of transfused erythrocytes)(19). At our clinic, leukocyte reduction of all blood components was implemented from 1995.

A number of clinical studies have documented the efficacy of leukocyte reduction in reducing the risk for febrile, non-hemolytic transfusion reactions (FNHTRs), refractoriness to platelet transfusion secondary to HLA alloimmunization, and

transmission of cytomegaloviruses (CMV). Other possible beneficial effects have been suggested, as reduced risk of transmission of cell-borne viruses, bacteria (90) and prions, prevention of adverse immunomodulatory effects (91-94) and decreasing the risk of TA-GVHD (95).

Febrile non-hemolytic transfusion reactions (FNHTRs) after transfusion are reduced, as shown in several studies, with reported reaction rates from 0.33% to 0.37% to 0.18 0.19% ($p < 0.001$). Not only the leukocytes per se, but cytokines (IL-1, IL-6, IL-8, TNF- α) secreted from WBC has been implicated for RBC (96-98) as well as platelets (99-102), which makes pre-storage more advantageous than post-storage filtration, due to the benefits of early removal of the leukocytes (103).

Platelets carry HLA class I antigens only, but in order to trigger an immune response, presence of HLA-class II presenting leukocytes (B-lymphocytes, monocytes, dendritic cells) are required. Removal of leukocytes from blood components to a level below 5×10^6 /unit (104, 105) is therefore considered to prevent primary HLA alloimmunization and refractoriness to platelets (106-113).

Reduced risk of transmission of infectious agents, i. e. viruses associated with subsets of leukocytes, as for cytomegalovirus (114) and sepsis (115, 116) have also been reported to be possible benefits. Universal leukocyte reduction was introduced in UK to reduce the risk of variant Creutzfeld-Jacob disease (vCJD) transmission by transfusion (117).

Filtration to reduce leukocyte content of components is preferably performed prestorage, before they release breakdown products or cytokines (118), to enable process control, and to avoid the risks of adverse reactions caused by bed-side filtration (119-122).

An alternative for the leukocyte reduction of separate blood components is filtration of whole blood, a procedure that allows removal from more than one blood component with one single filter. With the commonly used filters, the platelets are removed along with the leukocytes. The use of polyurethane for manufacturing of filters has allowed the development of filters that selectively remove leukocytes from whole blood, but allow the platelets to pass through the filter (123, 124). Blood components (erythrocytes and platelets) prepared after filtration of whole blood with this type of filter has been evaluated in Paper I.

1.7 TRANSFUSION ASSOCIATED GRAFT VERSUS HOST DISEASE

Transfusion associated Graft versus host disease (TA-GVHD) is a complication to transfusion of immunocompetent cells, i e concomitant leukocytes, which involves fever, rash, gastrointestinal symptoms and bone marrow hypoplasia with pancytopenia. The symptoms appear 1-2 weeks after infusion, and have a high risk of mortality (80-90%)(125-127).

The condition was first described in mice, and requirements for GVHD were defined by Billingham as 1) the graft must contain immunologically competent cells, 2) the host

must possess important transplantation alloantigens which are lacking in the donor graft, so that the host appears foreign to the graft and therefore capable of stimulating it antigenically, and 3) the host must be incapable of mounting an effective immunological reaction against the graft (128). The first reports described TA-GVHD only in immunosuppressed individuals, following any blood component containing viable T-lymphocytes, but it has become evident that this condition can appear in immunocompetent recipients. The risk factors are transfusion from HLA -homozygous donors coincidentally haploidentical with the recipients (129, 130). This situation occurs infrequently when blood components are given from random donors, but is more common in populations with limited HLA-diversity, such as in Japan, where many cases of TA-GVHD has been described (131). Transfusing blood components from related donors, and platelets from HLA-matched donors, means a high risk of TA-GVHD (132, 133).

T-lymphocytes and NK-cells have been described to be involved in the pathogenesis, and cytokines are considered of importance in the process (126).

The required dose of lymphocytes to induce TA-GVDH in mice is 10^7 (134). For humans, the dose has not been entirely defined, but case reports indicate that TA-GVHD occurred after a dose as low as 10^4 lymphocytes/kg in a neonate (135). The viability which has been shown to decrease during refrigerated storage also has an influence on the risk. All cellular blood components convey a risk for TA-GVHD, which is described in susceptible patients after the transfusion of whole blood, red cells, platelets, granulocytes and fresh non-frozen plasma (136). There have been no reports of cases following the transfusion of cryoprecipitate or fractionated plasma products. Plasma that has been frozen is generally not considered as giving risk for TA-GVHD, although a single case has been reported in a severely T-cell immunodeficient patient (137).

Based on these data, the major indications for prevention of TA-GVHD are for patients undergoing transplantation with hematopoietic stem cells, Hodgkin lymphoma (due to defective T-cell function), patients treated with fludarabine (purine analogue), intrauterine transfusions and in transfusions to premature neonates, and transfusions from relatives or HLA-matched donors.

No specific treatment for TA-GVHD has been proven to be successful in reversing an established extensive TA-GVHD, so the focus is instead on prevention. The current use of leukocyte-depleting filters achieves an amount of $<10^6$ leukocytes/unit. This level has been reported to reduce the risk of TA-GVHD substantially (95) but cannot be considered as a safe single method to protect from TA-GVHD, as the probable dose of lymphocytes to induce a risk for TA-GVHD is below that limit (138).

Gamma irradiation was the first method to be practiced, and is still the most prevalent method for prevention of TA-GVHD. Later, blood irradiation equipment using X-ray technology was made available for Blood Transfusion Services.

Methods to inactivate viral and bacterial pathogens in blood components have proved to be an alternative to irradiation for prevention of TA-GVHD. Novel psoralens, e.g. S59; amotosalen (INTERCEPT) has been synthesized to enhance viral and bacterial inactivation without compromising the platelet function. In a study performed by Grass et al (139), T-lymphocytes were shown to be exquisitely sensitive to S59. They were

inactivated to an undetectable level by a 3000-fold lower dose than acquired for the inactivation of pathogens. Other pathogen reduction systems, as riboflavin (Mirasol) has also been shown to inactivate lymphocytes to a degree which makes them suitable for TA-GVHD-prevention (140).

1.8 IRRADIATION OF PLATELETS

Irradiation, either gamma or X-ray, produces ionizations and free radicals that damage cellular DNA, thereby precluding replication required to allow the development of TA-GVHD. Specifically, it prevents T-cell replication following a dose sufficient to cross-link or damage T-cell DNA. Because erythrocytes and platelets are anucleate cells without DNA, doses sufficient to inactivate T cells does not seriously affect erythrocyte or platelet function (141). The irradiation devices display significant variation in dose distribution throughout the irradiated volume, e.g. 30% variation along the central axis and 35% between the periphery and the centre of the canister. Due to the uneven distribution of irradiation observed, the recommendation is to deliver a central dose high enough to ensure that the minimum dose also is sufficient to inactivate lymphocytes, the minimum dose required considered to be 25 Gy. This level is based on data from a study where red cell units were exposed to increasing doses of radiation, showing that doses of 15 Gy and 20 Gy abrogated most, but not all, clonal T-lymphocyte proliferation, whereas irradiation with 25 Gy abolished cell growth (142). According to European guidelines (13), the recommended dose is 25-50 Gy in any part of the irradiated canister.

Gamma irradiation equipment contains a radioactive source, either cesium (^{137}Cs) or cobalt (^{60}Co), that undergoes a constant rate of decay. Equipment with cesium 137 is the most widely used, due to its longer half-life (30 vs. 5.2 years). A longer half life is beneficial both for less frequent dosage adjustments as well as longer usable life of the source. Irradiators must be dose mapped annually, and dose adjustment at least annually for cesium sources, or quarterly for cobalt sources (142). Cesium irradiators typically allow 1-4 blood units to be irradiated at the same time. When loading the canister, the operator is protected by a lead shield, and the canister is rotated into position to be exposed to the cesium sources in the interior of the equipment. An irradiation indicator is often applied on the blood components, to indicate effective irradiation of each batch. Cesium is a highly reactive compound, hence the use is strictly regulated. High demands on security regarding the use of high activity sealed radioactive sources (HASS), concerning access, training, control of absorbed dose and leakage, and disposal are laid down in legislation (143). However, concerns about the safety, and reported dispersal accidents (144) from disposed devices has encouraged the development of an alternative to cesium irradiators.

In recent decades, X-ray equipment for blood components has become available, that uses 1-2 X-ray sources. In terms of safety, it is an advantage that X-rays are emitted only during irradiation cycles, and can be disposed of as any electronical equipment. However, these devices are considered to have higher cost for maintenance and part replacement costs, such as X-ray bulbs, which burn out periodically and need replacement. Control of absorbed dose is recommended at least 2 times per year, and control of radiation leakage during operation shall be performed regularly. Typically,

1-3 blood units can be placed in a canister and irradiated in one cycle. The use of irradiation indicators adjusted for X-rays are recommended.

Irradiation affects cellular membranes and results in potassium leakage in erythrocytes, which limits the storage after irradiation (145). However, limitation of storage time for irradiated platelet is not considered to be required, as platelets have a shorter shelf-life than erythrocytes.

A number of studies have been done to assess the effects of gamma irradiation of platelets, including in-vitro parameters and in-vivo recovery and survival, the majority indicating that irradiation does not compromise the quality (146-152). According to guidelines in UK, platelets can be irradiated with 20-50 Gy at any time during storage and thereafter be stored up to their normal shelf life after collection (153). Prestorage irradiation is preferred at many centers, mainly of logistic reasons, in order to have platelets readily accessible, which is especially important in emergency situations.

The studies outlined in paper IV address the effects of prestorage irradiation of platelets during storage for 7 days, which confirms the results from previous studies. In addition, evaluation of effects of X-ray irradiated platelets was performed, with similar results, indicating that X-ray do not compromise the in-vitro quality of platelets during storage for 7 days.

1.9 QUALITY ASSESSMENT OF PLATELETS

When introducing new methods for preparation and storage of platelets, assessment of the impact of platelets is essential.

In transfusion medicine, the main application of in vitro tests has been to determine platelet quality in PCs. The major disadvantage is that it is somewhat unclear how these results translate to platelet recovery and survival in vivo after transfusion. However, the in vitro data provide a level of reassurance prior to the clinical use of the component, whereas poor in vitro performance would be a major cause of concern and require further studied before introducing the method in routine production.

The hierarchy of studies in validation of major changes of methods are 1) in vitro tests 2) recovery and survival in healthy volunteers 3) count increment studies in thrombocytopenic patients, and 4) an assessment of haemostasis (measurement of bleeding) in thrombocytopenic patients (154).

Swirling, a light scattering phenomenon attributable to the discoid shape of non-activated platelets, is a morphological test which is non-invasive and easy to perform, and is used as a pre-issue test at many centers. Platelet concentrates that fail to swirl have pH below 6.4 or above 7.6 in most, but not all, cases (155).

For the metabolic parameters, it is known that pH values below 6.0-6.2 are associated with poor in vivo recovery (35, 156). The relationship between high pH and in vivo viability is less clear. Some studies demonstrate a loss of recovery in vivo at pH values above 7.2-7.7 (157, 158), whereas others show that PC with a pH up to 7.6 did not result in loss of in vitro function or poor in vivo recovery (159).

Extent of shape change (ESC) which is a measurement of the shape change induced by ADP by an aggregometer has been shown to correlate well with recovery in vivo (74). Hypotonic shock response (HSR), which is the ability of platelets to extrude water after the swelling caused by a hypotonic environment, is measured by an aggregometer. This parameter was shown to correlate to some degree with in vivo viability (74), although later studies could not confirm this (160). It was also shown by Holme et al, that ATP levels below $4.0\mu\text{mol}/10^{11}\text{PLT}$ causes loss of in vivo viability (73). Examples of substances released from α -granules, e.g. RANTES and PF4, are measured both as markers of activation, and for their purported role in allergic transfusion reactions (161).

In addition, expression of platelet glycoproteins involved in adhesion and aggregation (e.g. GPIb, GPIIa/IIIb), expression of activation markers (CD62P, CD63, CD40L), exposure of negatively charged phospholipids and formation of platelet-derived microparticles can all be studied during storage with flow cytometry using a number of commercial reagents (154).

Different in vitro test indicating apoptotic changes have also emerged, e.g. depolarization of mitochondrial membrane potential and microparticle generation (162). However, apoptotic changes during storage of platelets seem to play a role only in case of buffer exhaustion with consequent deleterious pH decrements (163).

The survival and recovery of autologous radiolabelled platelets transfused to healthy volunteers are considered to be the best indicators of PC quality, although the methods are technically demanding and laborious. A standard has been proposed by Murphy et al (164) that the mean recovery should be $>66\%$ and survival $>50\%$ of fresh platelets. Recently, a multicolor flow cytometry method was developed for simultaneous studies of recovery, survival, and function of transfused PLTs was described as an alternative method (165).

In clinical studies measurements of platelet count increments (CI) at 1 hour and 24 hours post transfusion are used to evaluate the effect of transfusions. A corrected count increment (CCI), takes the transfused dose and body surface of the patients into account. To agree with Cardigan et al (154), the platelet count provides little information of the functionality of the cells, but it is clearly a prerequisite for platelets to be able to circulate in order to function. Although, it may be argued that in the setting of therapeutic platelet transfusion (i.e. to stop a bleeding) the platelets, although well-functioning may not lead to a high increment, as the platelets are engaged in the hemostatic process, rather than circulating.

Another optional analysis, is assessment of platelet function in the context of clot formation, i.e. by use of thrombelastography (TEG, ROTEM)(166). Studies have shown that clot strength is minimally affected after storage of PC for five days (166, 167). A similar method described by Tynngård et al, is free oscillation rheometry (168, 169).

1.10 PLATELETS FOR TRANSFUSION

Platelet transfusions were shown to reduce mortality from hemorrhage already in 1960s (170), and the utilization has since increased steadily, to become a prerequisite for treatment in many clinical settings. Stroncek reported in 2007 that 2 million platelets were transfused in USA and 2.9 million in Europe (171). In Sweden, the demand for platelets is increasing every year, and during 2011, 50.000 units were transfused (19), or 5.3/1000 inhabitants, which is an increase of approx. 60% compared to 2000.

In order to meet the demands, hospital blood banks need to have platelets in store, readily accessible in acute situations. Due to the relatively short shelf-life of 5 -7 days, it requires careful planning in order to balance outdating and shortage.

To ensure proper storage conditions, platelets are stored on flat-bed shakers in incubators with regulated temperature. Continuous surveillance of temperature, and a system for alert when the temperature is out of range, needs to be in place. Conditions during transport of platelets should be validated, and monitored at regular intervals.

Regular quality controls with regard to cell content (platelets, leukocytes) and pH at the end of storage shall be performed, to ensure that requirements by current standards are met (13).

Although bacterial screening is mandatory only for prolonged storage of 7 days, it is performed on an increasing number of platelet in Sweden (19). Previously, at the time the studies in this thesis were performed, aerobic and anaerobic bacterial cultures were done, of approx 20% of platelets. At present, eBDS is performed, of approx 66% of platelets.

In Sweden 2011, all platelets were leukocyte reduced, and approx. 55% were irradiated. At our clinic, all blood components are leukocyte reduced. In addition, all platelets are irradiated, a praxis that was introduced due to the fact that the group of patients that need platelets overlap to a great extent the group that needs irradiated blood components. This fact made it an advantage from a logistic point of view.

Coding and labeling of blood components are necessary to ensure traceability, from the donors to the recipient, and also facilitates records of all sequential steps in preparation. With regard to platelets, a change of component code is made and recorded in connection with pooling of buffy coats to a platelet concentrate, leukoreduction, irradiation and bacterial screening etc.

Statistics from the Swedish Society of Transfusion Medicine (19) regarding number of platelet concentrates transfused, bacterial screening performed and positive bacterial cultures in Sweden 2000-2011 are shown in Figures 6-8.

Totalt antal transfunderade trombocytdoser per 1000 invånare och år 2000-2011

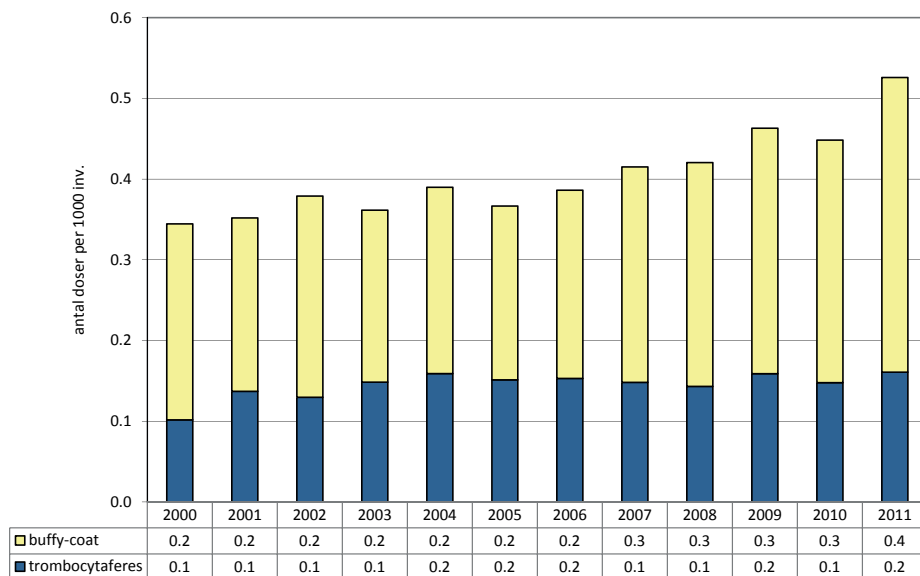


Figure 6. Total number of transfused PC per 1000 inhabitants in Sweden 2000-2011

Kontroll av bakterieförekomst i trombocytenheter

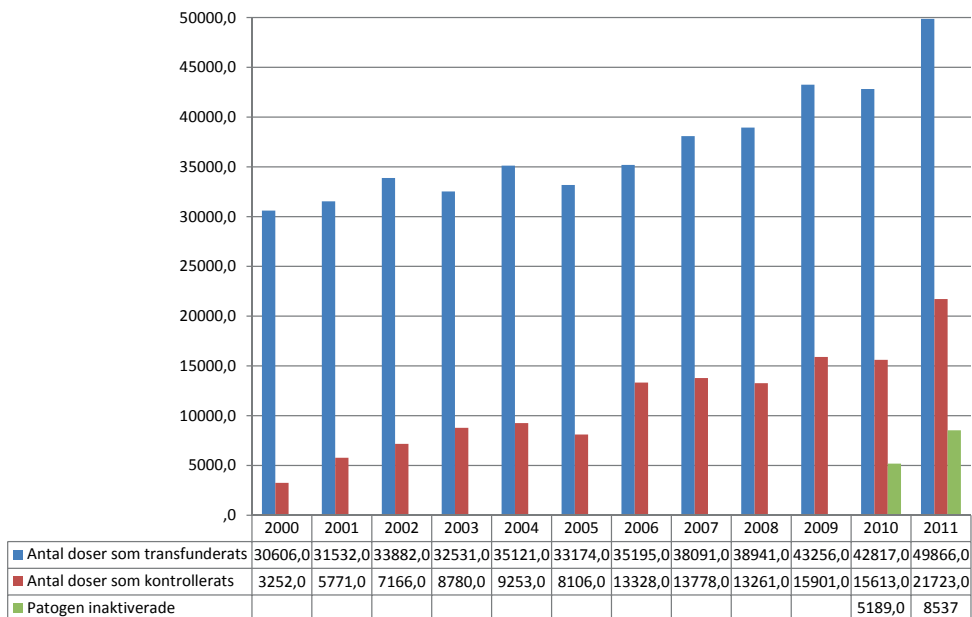


Figure 7. Bacterial screening of PC 2000-2011

Positiva bakterieodlingar i trombocytenheter

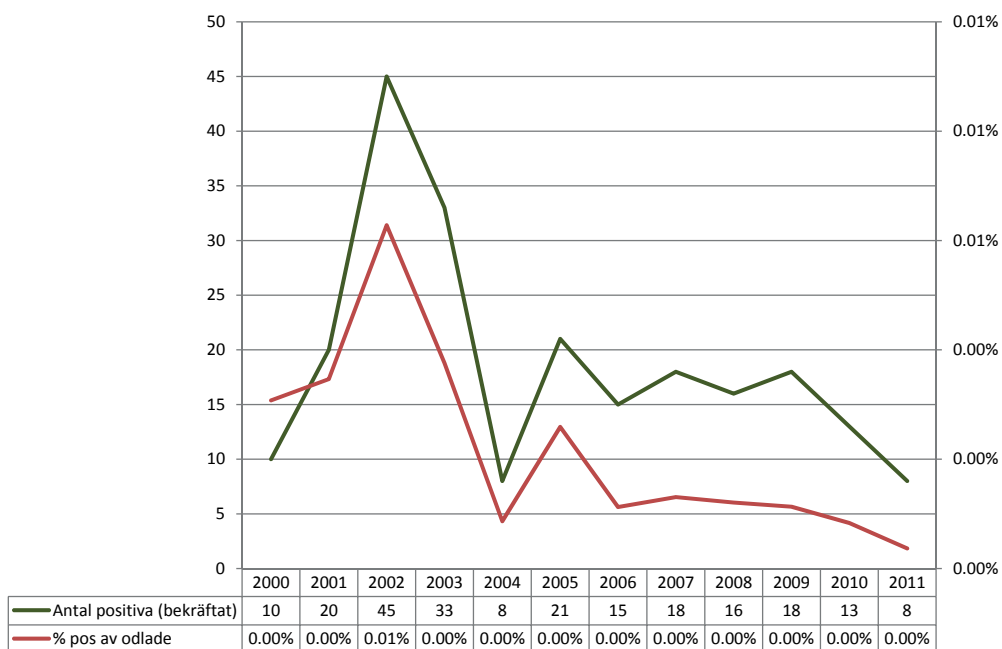


Figure 8. Number of positive bacterial cultures of PC 2000-2011

Figures 6-8. Marja-Kaisa Auvinen, based on statistics from Swedish Society of Transfusion Medicine 2011

2 AIMS

The overall aim is to gain increased knowledge of the impact of new equipment and new preparation techniques for blood component preparation, with special focus on improving quality and safety of platelets intended for transfusion.

The specific aims are:

Paper I – to study the impact of a novel platelet saving whole blood filter on prepared erythrocytes and platelet concentrates from pooled leukocyte reduced buffy coats during storage.

Paper II – to study the use of novel automated equipment for preparation of pooled buffy coats, with special attention paid to reduced platelet count variation due to selection of buffy coats based on donor platelet counts.

Paper III – to study the impact of different suspension media on the quality of platelets from pooled buffy coats and from apheresis, with focus on the effects of phosphate.

Paper IV – to study the impact of irradiation on different platelet preparations using both cesium radioactive source (gamma irradiation) and the novel X-ray irradiation equipment.

3 MATERIAL AND METHODS

3.1 PREPARATION AND STORAGE OF PLATELETS

Paper I

Whole blood was connected sterilely to a leukocyte depletion filter (prototypes, WBFSP, Terumo) made of multiple polyurethane sheets which allow penetration of platelets due to the characteristics of the plastic material (172). Filtration was immediately followed by centrifugation at 2700 g for 10 minutes at 22°C (Beckman J6MI centrifuge, Beckman instruments, Palo Alto, California, USA) and separation into RBCs, plasma and leukocyte-reduced BC using automated equipment (T-ACE, Terumo) and channeled side-flow quadruple bags (supplied by Terumo, made of PVC with DEHP as plasticizer). This is a variation of the top-and-bottom blood bag system. RBCs were suspended in 100 mL of SAGMAN solution. The leukocyte-reduced BCs were kept at room temperature overnight, and 5 BCs were pooled the following day by sterile connection into a pooling bag (600 mL, JMS, Singapore). The pooled BCs were rinsed with 300 ml platelet additive solution (T-Sol, Baxter, La Châtre, France) and centrifuged, thereafter the platelet-rich supernatant was transferred to a storage container (1000 mL, UPX-80 JMS, Singapore). The ratio of plasma: T-Sol in the platelet suspension medium was approximately 35:65. Platelets were compared to platelets prepared manually from pooled buffy coats with the same suspension medium. Platelets were kept at flatbed agitators at 20-24°C during storage for 7 days, and samples were taken at D1, D3, D5 and D7.

Paper II

Whole blood (450±45 mL) was drawn from healthy donors, in blood container systems containing 63 mL of standard CPD-solution as anticoagulant. The whole blood containers were kept at room temperature (20°-24°C) by cooling plates, and centrifuged at 2700g for 10 minutes at 22°C within 8 hours of collection. Centrifugation was immediately followed by separation into RBC, plasma and BC, using automated equipment. The BC, were kept overnight without agitation at room temperature (20°-24°C). In all experiments, the preparation of BC for platelets was similar.

In a first sequence, ABO-identical BC (5-6/pool) and PAS are pooled in a ring-shaped container. This step includes washing of the BC containers with PAS. In a second sequence, the contents of the ring-shaped pooling container are mixed and centrifuged, followed by transfer of the platelet-rich supernatant into a container placed in the center of the centrifuge. In the first design, the leukocytes in the platelet-rich supernatant was reduced by filtration after removal of the disposable from the equipment (used in experiments 1-3). In the next design, a leukocyte reduction filter and platelet storage container was integrated in the system (used in experiments 4-6). The suspension medium was a combination of platelet additive solution (approx. 65%), together with plasma carry-over (approx. 35%) from the individual buffy coats.

Platelets were kept at flatbed agitators at 20-24°C during storage for 7 days.

Bacterial cultures were performed on approx. 20% of all platelets, according to the standard routines on samples taken at the completion of component preparation.

Experiment 1: Paired in vitro study of BC-PCs (n=6) from 6 BC, suspended in a mix of CPD plasma carry-over from the individual buffy coats together with PAS-II, with the proportions plasma:PAS-II 35%:65%, where automated (OrbiSac) and manual preparation was compared.

Experiment 2: Automated routine preparation of PCs from pools of 6 BCs (n=409), for clinical use. Platelet count was determined in all units, and the recovery of platelets in some of the units (n=82).

Experiment 3: Standardization of platelet content
PC (n=98) intended for clinical use, prepared from 6 ABO-identical BC by the OrbiSac system prepared as described above. The selection of BC for pooling was made manually, on basis of the platelet concentration of the individual blood donors. Platelet count was determined in all units

Experiment 4: Paired in vitro study of PC from pools of 6 BC (n=6) using the OrbiSac system with an integrated leukocyte filter comparing two different storage containers, a Gambro ELP (Gambro BCT, Lakewood, CO, USA) and UPX-80 (JMS, Singapore) storage container.

Experiment 5: PC (n=611) intended for clinical use were prepared from 6 ABO-identical BC by using the OrbiSac system with an integrated leukocyte reduction filter. The platelet count was determined in all units, and the recovery of platelets in some of the units (n=24).

Experiment 6: Standardization of platelet content and reduction of number of BC in the pools

PC (n=292) intended for clinical use were prepared with the OrbiSac system that included an integrated leukocyte reduction filter, where pools of five ABO-identical BC were combined, using a computer program ("Orbiselect"). The platelet count was determined in all PC, and the variation in the platelet content was compared to that in PC prepared after pooling of the BC at random. The recovery of platelets was determined in a smaller number (n=36) of PC.

Paper III

The platelet preparations that are evaluated in this study are from pooled buffy coats and from apheresis, suspended in a mix of plasma and platelet additive solutions, one without phosphate (PAS-II) and one with phosphate (PASIII), or solely in plasma. All units were leukocyte reduced. Platelets were kept at flatbed agitators at 20-24°C during storage for 7 days.

Experiment 1: Paired study of platelets (n=6) prepared from 5-6 pooled buffy coats, suspended in a mix of CPD plasma and two different PASs (PAS-II and PAS-III), with the proportions plasma:PAS 35%:65%. Preparation was done by manual methods.

Experiment 2: Single study of platelets (n=8) prepared from 5-6 pooled buffy coats, suspended solely in CPD plasma.

Experiment 3: Paired study of platelets (n=7) prepared from 5-6 pooled buffy coats, suspended in a mix of CPD plasma and two different PASs (PAS-II, PAS-III), and addition of glucose, with proportions plasma: PAS: 20%:80%. Preparation was done by manual methods.

Experiment 4: Two apheresis systems were tested in two parallel studies.

Paired study of apheresis platelets (n=6) from single donors were collected with Amicus (Baxter), where platelets suspended in a mix of ACD plasma and PAS II, with proportions plasma: PAS 35%:65% were compared to apheresis platelets suspended solely in ACD-plasma.

Paired study of apheresis platelets (n=6) from single donors collected with Spectra (Cobe), where platelets suspended in a mix of ACD plasma and PAS II, with proportions plasma: PAS 35%:65% were compared to apheresis platelets suspended solely in ACD-plasma.

The apheresis collections were performed according to company equipment manuals.

Experiment 5: Apheresis platelets from single donors (n=14) were collected in three different blood cell separators: Spectra (Cobe), Excel (Dideco) and CS3000 Plus (Baxter), and suspended in a mix of ACD plasma and two different PASs (PASII, PASIII), with proportions plasma: PAS 35%:65%. The apheresis collections were performed according to company equipment manuals.

Paper IV

Apheresis platelets:

Platelets from healthy donors was drawn by apheresis equipment TRIMA (Caridian BCT, Inc, Lakewood, CO, USA) with in-process leukoreduction (LRS-chamber), and suspended in ACD-plasma from the donor. Initially, apheresis platelets suspended in 40% plasma and 60% PAS were prepared, but later replaced by apheresis platelets in 100% plasma.

Platelets from pooled buffy coats:

Whole blood (450±45 mL) was drawn from healthy donors, containing 63 mL of standard CPD-solution as anticoagulant. The whole blood containers were kept at room temperature (20-24°C) by cooling plates, and centrifuged at 2700g for 10 minutes at 22°C within 8 hours of collection. Centrifugation was immediately followed by separation into red blood cells (RBC), plasma and buffy coats BC, using automated equipment (T-ACE, Terumo or Optipress, Fenwal). BC, consisting of approx. 20 mL of blood cells and 25 mL of plasma, were kept overnight without agitation at room temperature (20-24°C). BC-PC was prepared by the OrbiSac system (Caridian BCT, Inc, Lakewood, CO, USA). Five to six BC and 300 ml of PAS-II (T-Sol, Baxter) were used per platelet unit prepared, with 35% plasma from plasma carry-over from BC and 65% PAS. The platelet units were kept on a flat bed agitator in a temperature-controlled cabinet (20-24°C).

The different platelet preparation techniques and the two different irradiation equipments have been evaluated, in paired studies:

Irradiation equipment with cesium as radioactive source:

Platelets prepared by apheresis, suspended in T-Sol/plasma, were divided into two equal parts (n=6), of which one part was irradiated the day of preparation, and the other half stored as non-irradiated reference.

Platelets prepared from pooled buffy coats, suspended in T-Sol/plasma, were pooled and subsequently divided into two equal parts (n=6), of which one part was irradiated the day of preparation, and the other half stored as non-irradiated reference.

Irradiation with X-ray equipment:

Platelets prepared by apheresis, suspended in T-Sol/plasma, were divided into two equal parts (n=6), of which one part was irradiated the day of preparation, and the other half stored as non-irradiated reference.

Platelets prepared by apheresis, suspended in plasma, were divided into two equal parts (n=6), of which one part was irradiated the day of preparation, and the other half stored as non-irradiated reference.

3.2 BACTERIAL SCREENING OF PLATELETS

Aerobic and anaerobic bacterial cultures on samples from the platelet units (by sterile docking of a sampling pouch) were performed at the Microbiological laboratory, Södersjukhuset, Huddinge University Hospital and Karolinska University Hospital.

3.3 IRRADIATION OF PLATELETS

Paper I-III

The platelets described in these studies were not irradiated. At the time of these studies, irradiation was performed post storage, on demand, before issue of the platelets.

Paper IV

Irradiation was performed immediately after preparation, either by Gammacell Elan 3000 (MDS Nordion, Canada) with cesium-137 as radioactive source, or by X-ray equipment Raycell (Best Theratronics, former MDS Nordion, Canada). Irradiation indicator, i.e. labels which by physical change indicates that irradiation has been performed, was used. The absorbed dose was minimum 25 Gy and maximum 50 Gy in any part of the container, according to European regulations (13). The absorbed dose are controlled once (Gammacell Elan 3000) or twice (Raycell) yearly, by dosimetry performed by MD Anderson Cancer Centre, University of Texas, Houston, USA.

3.4 IN VITRO ANALYSIS

3.4.1 Cellular and metabolic parameters

Paper I-III

Cell counts in the in vitro erythrocyte and platelet storage studies were made using an automated cell counter (AC 920, Swelab Instruments, Stockholm, Sweden).

Residual white blood cells in the PC were counted, using a Nageotte chamber (173).

For the determination of pH, pO₂, pCO₂, and bicarbonate, a pH/blood gas analyzer was used (Model 1610, Instrumentation Laboratory, Lexington, MA, USA). Glucose and

lactate were analyzed by spectrophotometry, using commercial kits (Cat Nos 16 and 826, Sigma Chemical Co. St.Louis, MO, USA). A firefly luciferase assay (65) was used for the determination of adenosine triphosphate and total adenosine nucleotides. The extracellular potassium concentration (Paper I) was analyzed in the department of Clinical Chemistry at Södersjukhuset, using a Hitachi 917 equipment (Hitachi, Tokyo, Japan). Hemolysis (paper I) was determined by a photo spectrometric method (Varian DMS 100, wavelength 540 nm). The method for measuring adenylate kinase (ATP:AMP phosphotransferase, EC 2.7.4.3) as described by Olsson et al (174) was used as a marker of platelet disintegration. In paper II, lactate dehydrogenase, using enzymatic techniques was introduced as another marker of platelet disintegration.

Paper IV

Cell counts in the in vitro platelet storage studies were made by an automated cell counter (Medonic CA620, Boule Instruments, Stockholm, Sweden).

Residual white blood cell count (WBC) on day 1 was determined with a Nageotte chamber and a microscope (Zeiss, standard, Chester, VA, USA)(173). For the determination of pH, pCO₂, pO₂, bicarbonate, glucose and lactate a pH/blood gas analyzer was used (ABL 800, Radiometer, Copenhagen, Denmark). Bicarbonate (mmol/L) was calculated from pH and CO₂ partial pressure measurements. The extracellular lactate dehydrogenase (LDH) activity (% of total), a marker for disintegration of PLTs, was measured with a spectrophotometric method (Sigma Aldrich kit 063K6003; St Louis, MO, USA; Spectrophotometer Jenway 6500, Staffordshire, UK)(175).

Analysis of the chemokines PF-4 and RANTES was performed with commercial kits in accordance with the manufacturer's recommendations (Quantikine R&D system, Abingdon, UK for RANTES, and Asserachrom Diagnostica Stago SA, Asnières, France for PG4) described in detail in earlier work (42).

3.4.2 Functional parameters

Paper IV

Hypotonic shock response reactivity (HSR) as well as the extent of shape change (ESC) measurements was performed using a dedicated microprocessor based instrument (SPA 2000, Chronolog, Havertown, PA, USA) with the modifications of these tests described by Van den Broeke et al (176). The total adenosine triphosphate (ATP) concentration, was determined with a Luminometer (Orion Microplate Luminometer, Berthold Detection Systems GmbH, Pforzheim, Germany) on the basis of principles described by Lundin (177).

3.5 STATISTICAL ANALYSIS

Mean and standard deviations are given. Groups are compared on a one-to-one basis, in paper I, II and III using a two-tailed t-test for a two-sample test, with statistically different significance at $p < 0.05$. In paper IV, a repeated measure ANOVA including post hoc test Bonferroni's adjustment was performed, with "Days" as the repeated factor and "Group" as a between factor. The P -value represents the difference between groups at specific time points and was considered statistically significant at $P < 0.001$. The analyses were carried out using the Statistica software, version 9 StatSoft, Inc 1984-2007 (SPSS, Chicago, IL, USA).

4 RESULTS AND DISCUSSION

4.1 PAPER I

The practice of leukocyte reduction of blood components raised a concern about the significant numbers of cells that were lost in these processes, for erythrocytes often 10-20% in the BC and additionally 10-15% in the leukocyte depletion filter, in total 20-35%. With a whole-blood filter, which results in two leukocyte depleted blood components, i.e. RBCs and plasma, these losses can be reduced. However, available whole blood filters also remove platelets, which imply that there will be no alternative to the apheresis technique for the supply of platelet concentrates (PCs).

In the present study, a leukocyte depletion filter is evaluated that removes WBCs but not platelets, and produce three different leukocyte depleted blood components, RBCs, plasma, but also PCs. PCs could be prepared in a second step from either platelet-rich-plasma with no further loss of red cells, or alternatively after centrifugation from the thin buffy coat (BC) layer formed by platelets which have passed through the leukocyte filter. With regard to the composition of BCs, there is no need to go deep down into the red cell layer to remove granulocytes, since these cells are already removed by the whole blood filter. What would be needed is a stable blood cell layer to save and to transfer platelets for preparation of PCs. By this, it would be possible to reduce the amount of blood cells in the BCs and, consequently, also reduce the loss of red cells in the BC removal step.

The present study is based on filtration of 30 whole blood units using prototype filters, primarily to investigate the efficacy of white blood cell removal, loss of red cells and platelets and secondly, to investigate RBCs and PCs during storage.

The leukocyte removal is highly effective resulting in a residual WBC count well below the recommended limit (i.e. in Europe 1×10^6 WBC per transfused unit)(13). The recovery of 90% of red cells after filtration of whole blood is encouraging. This reduction is consistent with loss of volume in filter and tubing. In the case of RBCs, we recovered 73% of the red blood cells in the whole blood. For platelets, 81% of the cells are left after whole blood filtration, indicating loss of volume as well as trapping in the filter. In our study, 66% of the original platelet count in whole blood was recovered in the PCs.

The basic in vitro investigation of storage of RBCs (n=10; randomly selected) for 42 days suggest that pH and concentration of adenine nucleotides are similar to standard RBCs with BC removed and stored in SAGMAN solution, with the exception of day 42, when ATP levels were significantly higher, and the degree of hemolysis, which is stable at a level of about 0.2% throughout storage, in whole blood filtered units. Generally, hemolysis increases gradually during storage of RBCs. The difference observed may be associated with the early removal of WBCs in the present study. Previous studies indicate that red cells stored in the presence of WBC undergo increased hemolysis, since proteases discharged primarily from granulocytes at disintegration have a potent hemolytic effect (178). The levels of extracellular potassium in whole blood filtered units during storage are similar to those in previously described studies (179, 180).

Six units of PCs were prepared from BCs and platelet metabolism were studied during storage. In the *in vitro* study of PCs during 7-day storage, significantly higher values in whole blood filtered units compared with reference units were found for concentration of glucose and lactate. These differences observed from day 1 throughout storage may be explained by differences in storage time of whole blood and BCs preceding PC preparation associated with the different preparation techniques. However, no effects on adenine nucleotide levels were found, suggesting stable storage conditions and unaffected energy generation by platelet metabolism. The additional occasional differences (pH, pO₂, pCO₂, bicarbonate and adenylate kinase) are probably of no importance.

The conclusions from this basic preliminary evaluation of the platelet-saving in-line whole blood leukocyte depletion filter are that the results with regard to leukocyte removal, recovery of red cells and platelets as well as *in vitro* storage investigations are encouraging. This new type of filter offers a possibility of combining the efficacy of whole blood filtration with preparation of PCs from conventional whole blood units, and may simplify blood component preparation.

Our basic study was later followed up by a multicenter study (123) with a whole-blood filter of polyurethane, the surface of which shows high biocompatibility and very low attraction for platelets. Leukocytes were removed by the mechanism of sieving. The filter (Imuflex WB-SP, Terumo) had been registered in Europe, allowing clinical use of the blood components prepared.

Average platelet recovery from WB to PCs of 62±10% compares well with previously published data on methods ensuring high platelet harvests (16, 181). Also, median Hb recovery of 81% compares favorably to reported recoveries in BC-removed and subsequently filtered erythrocyte concentrates (182-184), and preserved FVIII in plasma components.

Although the encouraging results with the prototype and Imuflex filter, this has not been pursued so far at our clinic, the main reason being the high costs of the filter.

4.2 PAPER II

Preparation of pooled buffy coat platelets, which until then was done by manual methods, was in this study performed with the new OrbiSac equipment. This equipment allows automation of the crucial preparation steps, i.e. rinsing of the buffy coat bags, and extraction of the platelet-rich supernatant into the final storage container. However, some of the preparation steps, as sterile docking of the buffy coat, and disconnecting the empty bags after rinsing, are performed manually. In the first period, the platelets were leukoreduced after completion of the OrbiSac process, by passing them through a leukoreduction filter. Later, a leukoreduction filter was integrated in the OrbiSac disposable kit, which enabled in-process filtration.

Another aspect of the preparation process addressed in this paper is standardization of platelet content in the final product. This is achieved by two means, i.e. selection of buffy coats for pooling, and by the higher reproducibility in processing when the

crucial processing steps (rinsing, centrifugation, extraction of platelet rich supernatant) are being automated. In contrast, when all processing steps are performed manually, the recovery of platelets is to a great extent dependent on the skill of the technicians. The selection of buffy coats for pooling was also a subject for investigation, with the aim to standardize the final platelet content. The platelet concentration in samples from the donor proved to be a useful tool to diminish the variability in final platelet counts, based on the assumption that the yield of platelets in the buffy coat from donors with high concentration would be higher, which is true provided that whole blood separation has been optimal.

Later, by including an in-process leukoreduction filter, which minimized the extra workload, the process was made more efficient. We also evaluated different storage containers, with regards to quality during storage over 7 days.

Taken together, the results show that standardization was achieved, the overall process resulted in platelets with high and stable recovery, with in vitro parameters indicating preserved viability during the storage period of 7 days. Bacterial cultures were performed on a large number of platelets, of which all were negative.

The OrbiSac system was introduced for routine preparation at our clinic as a result of this evaluation, and has been in use since then. Over the years, as the demand for platelets has increased, additional devices have been installed.

4.3 PAPER III

Based on earlier studies of PAS regarding the impact of citrate and acetate (76, 79, 80, 82), a PAS containing 10 mmol/L of citrate and 30 mmol/L of acetate, called PAS-II, was composed. PAS-II was introduced for storage of platelets prepared from pooled buffy coats, and for apheresis platelets at our clinic. BC-PC were prepared from whole blood collected with CPD as anticoagulant, whereas apheresis platelets were collected with ACD-A as anticoagulant. The apheresis procedure resulted in a platelet concentrate with high concentration, to which PAS-II was added, the proportion plasma to PAS being 40% to 60% (185). However, in vitro studies of these different platelet preparations, showed significantly lower values for adenine nucleotide levels, reduced consumption of glucose and production of lactate in apheresis PC using phosphate-free ACD as anticoagulant compared to BC-PC stored in phosphate-containing CPD. This indicates a difference attributable to the presence of phosphate. Platelet storage in PAS-II was compared with storage in a different PAS, designated PAS-III, which has a similar composition, except that phosphate is included as an additional component.

In experiments 1 and 2, BC-PC in three platelet storage media were compared, i.e CPD-plasma, a medium consisting of 35% plasma and 65% PAS-II and a medium consisting of 35% CPD-plasma and 65% PAS-III. The consumption of glucose and production of lactate was significantly higher in PAS-III than PAS-II, although no significant differences in ATP and total adenine nucleotide content was noted. The levels of adenine nucleotides were similar in undiluted CPD-plasma. The significantly higher levels in plasma with regard to pH and bicarbonate as compared to PAS-II and PAS-III are probably related to plasma composition rather than metabolic differences.

Based on the metabolic parameters, the results suggest that PAS-II is comparable to PAS-III or undiluted CPD-plasma when BC-PC is stored in a suspension medium with 35% CPD plasma inclusion.

In experiment 3, BC-PC with only 20% plasma inclusion and two different PASs (PAS-II, PAS-III), with supplemented glucose, were compared. Pronounced differences in glucose consumption and lactate production, in combination with higher adenine nucleotide levels, were found with PAS-III. The levels of ATP on day 5 of storage was below $4.0 \mu\text{mol}/10^{11}$ platelets observed with PAS-II, which is associated with loss of viability (74). Hence, these findings differed from what was observed with a higher proportion of CPD plasma included, and may be attributed to the presence of an alternate energy source in plasma, possibly free fatty acids.

In experiment 4 apheresis PC in ACD-plasma and medium consisting of 35% ACD-plasma and 65% PAS-II were compared. Significantly higher glucose consumption, lactate production and adenine nucleotide levels were found for undiluted ACD-plasma than with PAS-II. To investigate whether these differences were related to differences in phosphate concentration, storage of apheresis PC using either PAS-II or PAS-III was studied in experiment 5. The results were similar to those in experiment 3, i.e. significantly higher glucose consumption and lactate production in combination with higher adenine nucleotide levels were found with PAS-III. Thus, the difference between the effects of the PASs in experiment 5 could only be related to the concentration of phosphate.

The conclusion based on the findings in these experiments is that the concentration of phosphate, like that of citrate or acetate, may have a significant influence on platelet metabolism when PASs are used. With respect to apheresis PCs using ACD as anticoagulant, our results suggests that the differences found is related to the concentration of phosphate. The presence of phosphate seems to be a critical factor to avoid low adenine nucleotide levels.

The results for BC-PCs using CPD anticoagulant suggest that PAS-III which contains phosphate in addition to citrate and acetate, is preferable to PAS II when the plasma included in the suspension medium is less than 35% of the volume.

4.4 PAPER IV

Gamma irradiation:

Paired studies of PCs from pooled buffy coats and from apheresis, stored in platelet additive solution/plasma, which were irradiated prestorage with cesium gamma irradiation equipment, showed similar in-vitro parameters compared to non-irradiated controls at all time-points during storage for 7 days. The in-vitro parameters were similar to those previously observed during storage of PCs prepared in the same way, and were all above the ranges associated with poor storage conditions (73).

X-ray irradiation:

For both groups of studied platelets, i. e. apheresis platelets, stored in platelet additive solution/plasma and in 100% plasma, and prestorage irradiated with X-ray equipment, the results are in the range previously reported for platelets prepared as in the present

study (186). None of the studied parameters proved any statistically significant difference between the groups at any time-point during storage for 7 days.

The present study confirms the findings of previous published studies on the effects of gamma irradiation by radioactive sources. A wide variety of platelet preparations have been included such as platelets derived from whole blood and apheresis with different additive solutions/plasma combinations and for various length of storage(187),(146),(147),(148-152). Although some diverging results regarding studied parameters have been reported, the majority of previous studies have not shown any major deleterious effects.

However, there are few studies on the effects of gamma irradiation by X-ray technique on blood components, and platelets in particular. The studies have focused mainly on red cells (188-190), and data indicate no significant difference between gamma irradiation and the X-ray technique on quality parameters. The present study includes X-ray irradiated platelets which have been stored for a prolonged period of time, which is an advantage from a logistic point of view.

The present results indicate that prestorage irradiation of platelets, with either X-ray or cesium based techniques, causes minor changes compared with non-irradiated controls, of which none were statistically significant. The studied parameters after irradiation are within the normal range, and meet the quality requirements at all time points.

Whether these similar in vitro results translate into a comparable clinical outcome may not be definitely answered. In a recent study (191) the corrected count increment (CCI) was analyzed after transfusion of fresh and stored buffy-coat derived gamma irradiated platelets in T-Sol to 60 allogeneic haematopoietic progenitor cell transplant recipients. Transfusion of fresh platelets resulted in a successful increment at both 1 and 24 hrs after transfusion. Transfusion of stored platelets resulted in a successful increment at 1 h after transfusion, but a less successful increment was noted at 24 h.

Taken together, this study concludes that pre-storage irradiation with both gamma and X-ray irradiation can be applied without compromising the in vitro quality of platelet preparations. Further in vivo studies are warranted however, to conclude to what extent these results corresponds with in vivo effects.

5 CONCLUDING REMARKS

This thesis has focused on new techniques for preparation of blood components, with special emphasis on platelets. The studies all represent new options for blood component preparation, which has enhanced efficacy and safety.

The major findings are

Paper I- Whole blood filtration with a leukocyte reduction filter that saves platelets achieved effective leukocyte depletion meeting current standards and satisfactory recovery after filtration. The storage characteristics for RBCs and PCs were similar to those of standard preparations. The whole blood in-line filter saving platelets is a new option for whole blood processing, which may simplify leukocyte depletion and blood component preparation.

Paper II- Automated preparation of PC from pooled buffy coats by the OrbiSac system with in-line leukocyte reduction filter is equivalent to the previous manual techniques regarding in vitro characteristics during storage for 7 days. The PCs are characterized by high recovery of platelets and sufficiently low leukocyte counts, and meet current quality criteria according to European standards.

The selection of buffy coats and the uniform recovery resulted in reduced variation of platelet content.

Paper III- The findings indicate that the concentration of phosphate, like that of citrate and acetate, has a significant influence on platelet metabolism when PASs are used, and is a critical factor to avoid low adenine nucleotide levels.

The results for BC-PCs using CPD anticoagulant suggest that PAS-III which contains phosphate in addition to citrate and acetate, is preferable to PAS- II when the plasma included in the suspension medium is less than 35% of the volume.

Paper IV- Prestorage irradiation of platelets, either with X-ray or cesium based techniques, causes minor changes compared with non-irradiated controls. The studied parameters after irradiation are within the range previously reported for platelets prepared with similar techniques, and meet the quality requirements at all time-points during storage up to seven days.

To what extent these results corresponds with the in vivo effects is still not entirely clear, and further in vivo studies are warranted.

As a final remark, the author wish to note, that during the time since the publication of these data further development has been done in the field, especially with regard to PASs and automation.

6 FUTURE PLANS

6.1 PATIENT STUDY

A platelet transfusion study has been started, with the aim to compare the clinical effects of platelets from pooled buffy coats stored in a new type of additive solution, PASIIIM, to those stored in the additive solution that has been used previously, PAS-II. The primary hypothesis being an increased platelet count increment after transfusion of platelets stored in PAS-IIIM compared to PASII due to decreased activation in vitro. PAS-IIIM has been used at our clinic since 2010, and is currently used in other European countries, and data from transfusion studies are warranted.

Patients treated with allogeneic hematopoietic stem cell transplantation with myeloablative therapy are included. Children (<50 kg), known alloimmunization to platelet antigens (HLA, HPA) will not be included.

The patients will receive either PAS-II the first time after the transplantation, and then PASIIIM, or reverse. Each patient is transfused at two occasions within the study. Samples will be taken before and after (1 and 24 hours) the transfusion of platelets to analyze platelet count increment and corrected count increments. The intervals between the transfusions will be recorded, in order to evaluate if this is increased after transfusion with the new PAS.

In addition, clot formation analysis of whole blood, by thrombocytelastography (ROTEM) will be performed before and (1 hour) after transfusion.

6.2 PNEUMATIC TUBE TRANSPORT

Pneumatic tube systems (PTS) reduce the workload and turnaround time of laboratories. Blood components, including platelets, should be of the highest quality possible, but so far, there is few data (192) regarding the effects on platelets by transport via a dedicated PTS, or if platelets age and number of runs has an effect. The aim of this study is to evaluate the in vitro effects of PTS transport on the quality of fresh, stored and returned platelets prepared from pooled buffy coats stored in approximately 70% PAS (PAS-IIIM).

6.3 WASHING OF PLATELETS

For patients with previous anaphylactic transfusion reactions, washing of platelet concentrates, either manually or with cell processors is performed to remove the suspension medium. This process has been evaluated by several authors, including platelet in-vitro parameters after washing with various solutions (193),(193, 194). A Japanese centre has recently evaluated washing of PLTs with Haemonetics ACP 215, a device used at our clinic for washing of erythrocytes. To use this equipment for washing of platelets as well is a new option, with the advantage of washing in a closed system, which may allow storage of washed PCs for more than a few hours after washing.

7 SVENSK SAMMANFATTNING

Blodtransfusion har under den senare hälften av 1900-talet kommit att få en allt större betydelse inom sjukvården. Laboratoriespecialiteten Transfusionsmedicin spänner numer över ett stort kunskapsområde, vari bl.a. ingår att välja ut lämpliga blodgivare och tappningsteknik, uppdelning av blodet i beståndsdelar (komponenter) och förvaring av dessa. Från en blodgivning kan blodet delas upp i röda blodkroppar, plasma och lättcellskoncentrat, där majoriteten av trombocyterna finns. Fyra till sex lättcellskoncentrat poolas för att framställa ett trombocytkoncentrat (transfusionsdos) till en vuxen patient. Trombocytkoncentrat kan också framställas med aferesteknik, genom att blodet från en givare centrifugeras kontinuerligt i en särskild utrustning, varvid trombocyterna avskiljs och samlas upp, och resten av blodet ges åter till givaren.

Sedan 1970-talet är uppdelning av helblod i komponenter den gängse rutinen, dels p.g.a. att det möjliggör att patienterna kan transfunderas med de celler som behövs, och dels p.g.a. att förvaring av de olika cellerna kan optimeras. Utveckling av centrifugeringsteknik och plastmaterial har varit av stor betydelse för att uppfylla krav på sterilitet, renhet och kvalitet hos de framställda komponenterna. De framställningsmetoder som används ska vara skonsamma, för att varken skada cellerna eller ha negativ inverkan på deras funktion.

Studier av de olika blodcellernas ämnesomsättning har lett fram till kunskaper om bättre förvaringsbetingelser, vilket inkluderat användande av tillsatslösningar för röda blodkroppar och trombocyter. Andra aspekter av blodkomponentframställning som utvecklats under de senaste årtiondena är metoder att reducera antalet vita blodkroppar i blodkomponenter och metoder att standardisera cellinnehållet i koncentrat av trombocyter.

Det finns många fördelar med att filtrera blodkomponenter för att avlägsna vita blodkroppar, t.ex. minskad risk för transfusionsreaktioner med feber, utveckling av antikroppar mot vita blodkroppar och överföring av vissa virus (t.ex. cytomegalovirus, CMV). I många länder har man infört filtrering av alla blodkomponenter, och i Sverige filtreras numer en majoritet av alla blodkomponenter.

För patienter med nedsatt immunförsvar, t ex. efter transplantation med blodstamceller, eller för tidigt födda barn under nyföddhetsperioden, finns det dessutom behov av att bestråla blodkomponenter innan transfusion, för att förhindra att medföljande vita blodkroppar leder till en immunologisk reaktion (s. k. graft versus host disease; transplanterat mot värd-sjukdom) som ofta har dödlig utgång. Bestrålning utrustning med cesium som radioaktiv källa har oftast använts, men under senare år har även röntgenutrustning anpassad för bestrålning av blodkomponenter blivit tillgängliga.

Undersökningarna i denna avhandling tar upp nya sätt att framställa blodkomponenter, med särskilt fokus på trombocyter:

1) Studie av helblodsfilter som avlägsnar vita blodkroppar men släpper igenom trombocyter, visar att tillräckligt låga nivåer av kvarvarande vita blodkroppar, och hög

andel av erythrocyter och trombocyter finns kvar efter filtrering, och har god kvalitet som bibehålls under förvaring.

2) Studie av automatiserad framställning av filtrerade trombocyter med OrbiSac, visar att metoden framställer trombocytkoncentrat med innehåll av trombocyter och kvarvarande vita blodkroppar som uppfyller gällande kvalitetskrav och bibehåller god kvalitet under förvaring. Genom att den automatiserade metoden ger reproducerbara utbyten av celler och genom att välja ut vilka lättcellskoncentrat som ska ingå i trombocytkoncentratet, kan man minska variationen i trombocytantal mellan de olika trombocytkoncentraterna.

3) Undersökning av effekter av fosfat i tillsatslösningar för trombocyter, har visat att fosfat är en nödvändig ingrediens i tillsatslösningar, för att bibehålla nivåer av adeninnukleotider som behövs för cellernas viabilitet.

4) Undersökning av effekt av gamma- och röntgenbestrålning på trombocyter har inte kunnat påvisa någon negativ effekt på trombocyter under förvaring upp till 7 dagar.

Det bör dock noteras att dessa laboratorieundersökningar (in vitro) bör kompletteras med undersökningar av blodkomponenternas effekt vid transfusion till patienter (in vivo).

Sammantaget ger dessa nya tekniker och tillsatslösningar möjlighet att öka effektiviteten och säkerheten vid framställning av blodkomponenter. Vidare utveckling har också ägt rum inom området, framför allt vad gäller tillsatslösningar och nya utrustningar för automatiserad framställning av blodkomponenter.

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